

From the Department of Medicine, University Hospital, Lund, Sweden.

CYTOLOGY OF TOXIC GOITER
as studied in fine needle aspirates

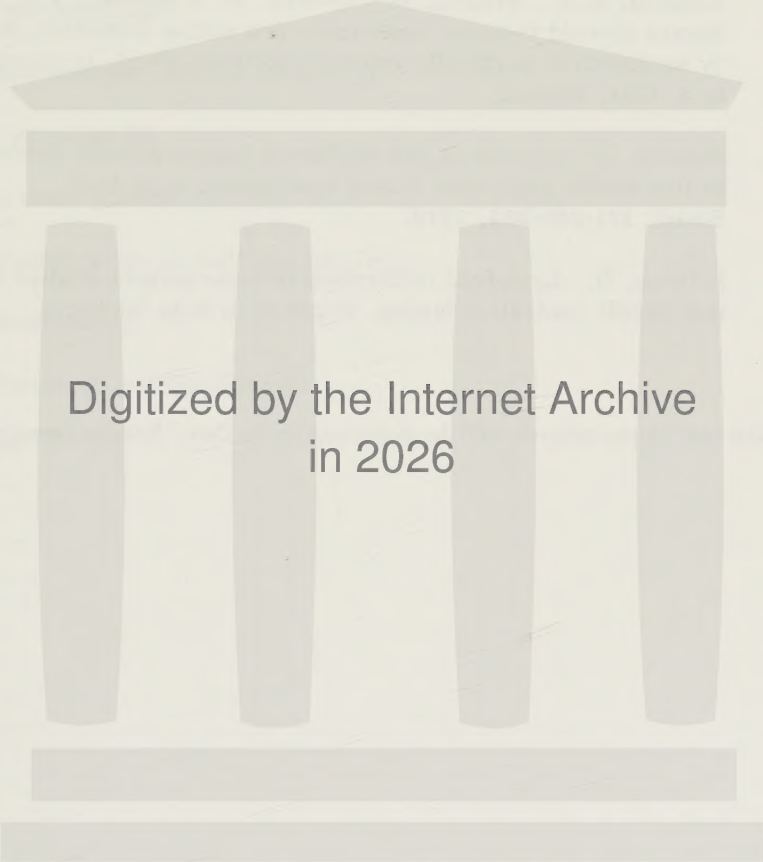
by

Göran Nilsson

Lund 1972



Printed in Sweden
Studentlitteratur
Lund 1972



Digitized by the Internet Archive
in 2026

The present dissertation is based on the following communications.

- I. Nilsson, G.: Nuclear size classes in fine needle aspirates from toxic goiters. In press in *Acta Endocr.*
- II. Nilsson, G.: Marginal vacuoles in fine needle aspiration biopsy smears of toxic goiters. In press in *Acta path. microb. scand.*
- III. Englund, N. E., Nilsson, G., Owman, S. & Sundler, F.: Human thyroid C-cells: occurrence and amine formation studied by perfusion of surgically removed goitrous gland. In press in *J. Clin. Endocr.*
- IV. Nilsson, G.: C-cells in non-malignant human goiters studied in fine needle aspiration biopsy specimens. *Acta Med. Scand.* 191:249-256, 1972.
- V. Nilsson, G.: Lymphoid infiltration in toxic goiters studied with fine needle aspiration biopsy. In press in *Acta Endocrin.*

In the text these papers will be referred to by their Roman numerals.

CONTENTS

Introduction

Material and methods

Nuclear size classes in toxic goiters (I)

Marginal vacuoles (II)

C-cells in toxic goiters (III, IV)

Lymphoid infiltration of toxic goiters (V)

General Discussion

General summary and conclusions

References

INTRODUCTION

The origin of increased thyroid hormone production in thyrotoxicosis - the hyperfunctioning thyroid cells - has been studied by the microscope for a century. The light microscopical picture of histological sections of toxic goiters is well recognized, though it is rarely seen in unadulterated form nowadays thanks to preoperative antithyroid treatment. The most characteristic feature of this picture is a tall follicular epithelium, often forming papillary projections into small follicular lumina containing scant colloid (Lindsay 1964). The cell nuclei are large and situated basally. Recent studies (Heimann 1966) have provided valuable information about the ultrastructure of toxic goiters. According to these studies, toxic goiters are distinguished from normal human thyroids by an especially well-developed endoplasmic reticulum with widely dilated cisterns. Furthermore, in toxic goiters the mitochondria are increased in size and number and the Golgi apparatus has a wider extension as compared with normal thyroids.

Descriptions of the cytomorphologic appearance of toxic goiters in fine needle aspirate smears are rare (Myren & Sivertsen 1962; Söderström 1966). Such aspirates consist of individual cells and cell groups selected from the thyroid according to the facility with which they are detached and carried away by the current of fluid initiated by aspiration with a fine needle with an outer diameter of 0.6-0.7 mm. This approach has the obvious disadvantage that the general tissue architecture cannot be appreciated, but, on the other hand, many cytoplasmic and nuclear details are usually much more distinctly visualised than in light microscopical histological sections. Furthermore, these aspirates are easily obtainable also from untreated toxic goiters and thereby they provide a unique possibility to study cytomorphology of such goiters uninfluenced by antithyroid medication. The aspirates can also be obtained before surgical, radioiodine, or long-term thiocarbamide treatment and may therefore be useful for selecting cases for different therapeutic measures.

The purpose of the present investigation was to evaluate possible clinical and pathogenetic implications of fine needle aspirates. Special attention was thereby focused on three questions.

1. Is cytological examination of goiter aspirates of any value for differentiating between toxic and non-toxic goiters?

2. Is cytological examination of goiter aspirates of any value for predicting recurrence of hyperthyroidism after long-term thiocarbamide treatment? This question is of great importance with regard to the choice between long-term thiocarbamide treatment and ablation of the toxic goiter by surgery or radioiodine.

3. Is cytological examination of any value in the investigation of the pathogenesis of thyrotoxicosis?

The investigation was based on a closer study of some observations made by the author during routine clinical examination of toxic goiter aspirates and thought to be of interest with regard to the above-mentioned questions. In the follicular epithelium nuclear size classes of the Jacobj type were studied with karyometric and cytophotometric methods. A special type of vacuoles, marginal vacuoles (MV) in the cytoplasm of the follicular epithelium, was studied for its value in differentiation between toxic and non-toxic goiters as well as to its theoretical implications. Lymphoid cells and C-cells in aspirate smears from toxic goiters were also investigated. Identification of the last-mentioned cell type necessitated extension of the investigation to include a study also of histological sections of L-DOPA (I-dihydroxyphenylalanin) perfused surgical specimens from toxic goiters.

MATERIAL AND METHODS

General presentation of the patient series

The investigation was based on 118 toxic goiters subjected to fine needle aspiration biopsy in the medical department A of the hospital of Lund during the years 1963-70. Goiters of different types were routinely examined with cytodiagnostic fine needle aspiration biopsy during this period. Hyperthyroidism was diagnosed on the basis of clinical examination and laboratory tests, including serum proteinbound iodine (PBI), in vitro triiodothyronine uptake (T_3 -test), or radioiodine uptake test of the thyroid. Since the material was partly analysed in retrospect, the laboratory and clinical examinations were not quite uniform through the whole series. The author had the opportunity of examining most of the patients personally. The remaining patients were examined by different physicians. Since not all of the patients were examined by one physician, comparison between different patients of some parameters such as goiter size and nodularity was considered unjustified.

All 118 toxic goiters were examined for lymphoid infiltration and C-cells. In the studies of nuclear size classes and marginal vacuoles only part of the material, was used, viz 52 and 49 cases respectively. By studying nuclear size classes (I) this restriction was made because the cellular yield occasionally did not permit a study of 1000 nuclei of the follicular epithelium and because of lack of determination in 59 cases of the long-acting thyroid stimulating factor. LATS, which proved essential in this connection. The difference between toxic and non-toxic goiters with regard to marginal vacuoles (II) could be clearly proved in part of the original series and further extension of the investigation in this field was therefore judged unnecessary. The patients of the latter series were selected as consecutive cases within the main series.

In order to evaluate the differential diagnostic importance of different traits in the cytological picture of toxic goiters comparison was done with series of benign non-toxic goiters without signs of thyroiditis in some parts of the investigation (I, II, III, IV). The euthyroid state of the patients in these series was secured by clinical examinations

and determinations of PBI and T_3 . Further details about these patients are presented in papers I, II, III and IV.

In order to evaluate the prognostic significance of different cytological findings with regard to thyroid function, those cases of thyrotoxicosis were particularly studied in which the presence or absence of recurrence of hyperthyroidism during at least 18 months after withdrawal of a long-term thiocarbamide treatment of 1-2 years could be estimated. This part of the material comprised 28 cases. In all these patients thyroid function was estimated from laboratory studies in all cases including PBI and T_3 -test. Furthermore, 24 of the above 28 patients were examined clinically in this hospital for recurrence of hyperthyroidism. In 4 cases information about the patient's thyroid status including the values of PBI and T_3 was obtained from physicians outside the hospital.

Laboratory findings

Serum protein bound iodine (PBI) as measured in a technicon Autoanalyser (Reiley & Gochman 1964) was found elevated in all 116 toxic goiter patients so examined.

In vitro triiodothyronine uptake test (T_3 -test) was performed with the aid of the Sephadex column method (Nosslin 1965) in 101 of the hyperthyroid patients and was elevated in 99. Like PBI, this test is done as a routine test in the chemical central laboratory of this hospital.

Thyroid uptake of radioiodine at 2 and 24 hours was determined as routine measures in 82 and 89 hyperthyroid patients, respectively. The thyroid uptake was elevated at 2 hours in 72 cases and at 24 hours in 83. Furthermore, the radioiodine turnover, measured as serum protein-bound iodine ($PB^{131}I$) after 48 hours in 50 cases, was found elevated in 45. Scintigraphy was performed in 78 cases. An evenly distributed uptake of radioiodine in the goiter was found in 43. In 2 cases a solitary toxic adenoma was found with suppression of iodine uptake in the rest of the thyroid. In the remaining cases different types of irregularities in radioiodine uptake were noted. The isotope studies were performed at the radioisotope laboratory, Department of Radiology in the hospital of Lund.

The long acting thyroid stimulating factor, LATS, was determined according to the Mc Kenzie (1958) method as modified by Rerup & Melander (1965) in the department of Pharmacology, University of Lund. LATS-activity was found in 15 of 59 hyperthyroid patients examined. The author would like to thank Drs C. Rerup and A. Melander for determination of LATS.

Thyroid autoantibodies. The passive hemagglutination technique was used for demonstrating antibodies against thyroglobulin. This test was positive in 21 of 54 hyperthyroid patients studied. Complement fixing antibodies were demonstrated in 10 of 54 patients so examined. The serological studies were done as routine performances in the Department of Bacteriology, University of Lund.

Biopsy procedure

The biopsies were performed as described by Söderström (1966) using fine needles with an outer diameter of 0.6-0.7 mm. In most cases a 10 ml one-hand aspiration syringe designed by Franzen (KIFA) was used but this was sometimes replaced by a disposable 10 ml syringe. Local anaesthesia was not used because the biopsy needle was roughly of the same thickness as the anaesthesia needle and would presumably have caused the same degree of discomfort as the biopsy procedure. The biopsy specimens were spread as smears, which were allowed to dry in the air.

Staining procedure

The smears were routinely stained with the May-Grünwald-Giemsa stain. A small number of specimens were also stained with hematoxylin-eosin and for acid phosphatase (Burstone 1958).

Cytophotometric measurement of Feulgen-DNA

Five aspirates were examined. Thereby smears were made on Bürker-slides which were dried in the air and fixed in absolute alcohol for 40 minutes. Hydrolysis was done in 1 N HCl for 8 minutes at 60° C. After Schiff staining cytophotometric measurements were made with a high resolution rapid-scanning microspectrophotometer (Caspersson & Lomakka 1962). The DNA-content of individual nuclei was determined at a wavelength of 546 Å. The polymorphnuclear leukocytes of the same smear were used as reference cells. For further details see I.

Determination of lymphoid cell content in the aspirates

In some parts of the investigation it was found necessary to objectify an increased occurrence of lymphoreticular cells in biopsy smears. For this purpose the quotient was determined between lymphoreticular cells and polymorphnuclear cells among 200 leukocytes in these smears. The corresponding quotient in smears from peripheral blood could be determined in 85 of the patients examined and was found to range between 0.2 and 1.3. For practical purposes an arbitrary limit was set at 1.75, which is well above the quotient found in peripheral blood. Above this limit an excess of lymphoreticular cells was considered as present.

NUCLEAR SIZE CLASSES IN TOXIC GOITERS (I)

It is generally accepted that with few exceptions the amount of deoxyribonucleic acid (DNA) per nucleus is constant in all somatic cells of the same species (Vendrely & Vendrely 1955; Hale 1963). This rule of nuclear DNA-constancy does not, however, imply a corresponding constancy of nuclear volume. Variation of this volume depending on fluctuations in nuclear water- and protein content has been shown to be intimately connected with essential cell functions such as RNA-synthesis (Harris 1967) and protein synthesis (Oehlert & Schultze 1960). Also in the thyroid, animal experiments have provided excellent illustrations to the combination of DNA-constancy and considerable fluctuations of nuclear volume during different functional conditions (Alfert & Kahn 1955).

The most striking exception to the rule of nuclear DNA-constancy is the occurrence in some organs, especially the liver, of a minor number of large nuclei, whose DNA-content falls into separate classes clearly distinguished from that of the bulk of the nuclei. The average DNA-content of the individual DNA-content classes tend to form a simple geometric series (1.2.4...). The DNA-content classes are reflected in corresponding nuclear size classes, the existence of which was pointed out in the classical works of Jacoby (1925, 1935).

The biopsy aspirates studied here provide a unique possibility to study variation of size and DNA-content of individual nuclei in toxic goiters uninfluenced by antithyroid medication. It was found that the nuclear size variation in such goiters was often characterized by the grouping of nuclei in different size classes giving rise to an apparent discontinuous anisokaryosis (fig 1). Thus most nuclei formed on distinct size class, while a few large nuclei formed on or two separate size classes. These findings most often quite evident at a glance in the microscope (fig 1), could be verified by nuclear diameter distribution curves based on measurements of 150-200 nuclei from each biopsy specimen (fig 2). The average diameter in the main peaks of these curves was larger in toxic goiters than in non-toxic goiters (Student's t -test = 6.10; $p < 0.001$) and normal thyroids ($t = 4.32$; $p < 0.01$). See fig 3. Cytophotometric measurements of the Feulgen-DNA-content of individual nuclei in five of the toxic goiter aspirates showed that nuclei in size classes distinct from the main class correspond to nuclei in separate DNA-content classes, the mean DNA-content of which approximately form multiples

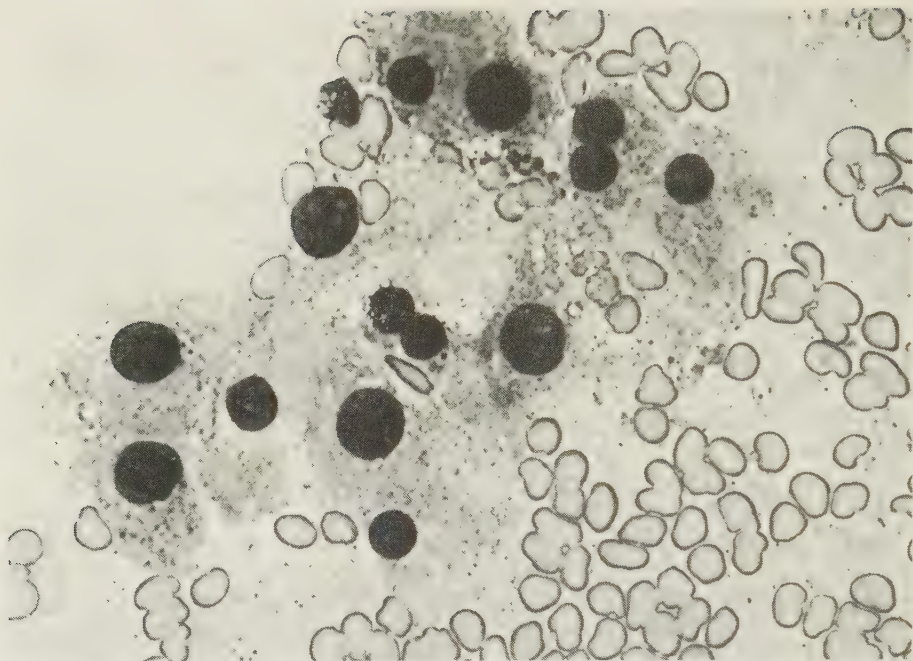


Fig 1. Nuclear size classes in fine needle aspirates from a toxic goiter.
x 160.

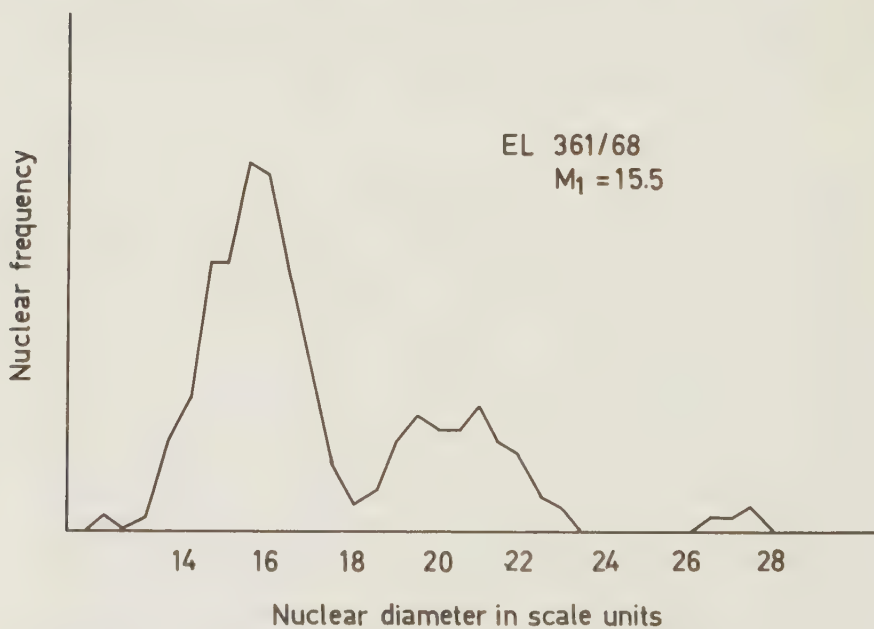


Fig 2. Nuclear diameter distribution curve in a toxic goiter.

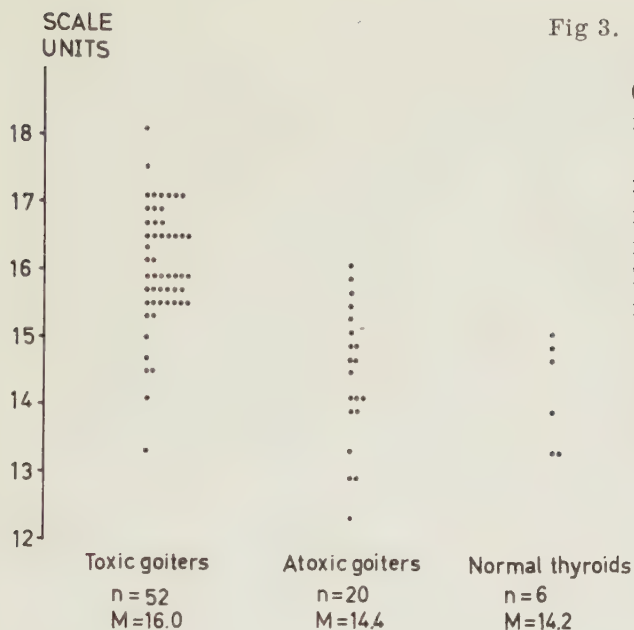


Fig 3. Mean diameter of the basic nuclear size class (M_1) in toxic goiters, non-toxic goiters without signs of thyroiditis, and normal thyroids. A significant difference was found regarding M_1 between toxic and non-toxic goiters ($t = 6.10$; $p < 0.001$) as well as between toxic goiters and normal thyroids ($t = 4.32$; $p < 0.001$).

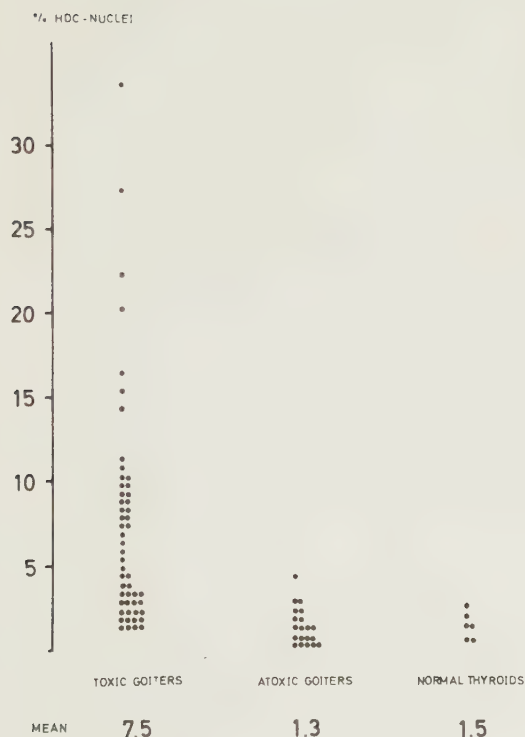


Fig 4. Frequency of HDC-nuclei in three types of thyroid gland. $P < 0.001$ for difference between toxic and non-toxic goiters. $P < 0.001$ for difference between toxic goiters and normal thyroids (rank-sum test).

of the mean nuclear DNA-content in the basic class.

At this point the author had to face a problem of terminology. Thus large nuclei of the above-mentioned type, forming separate DNA- and size classes, are most often called polyploids. However, in the terms of the cell cycle (Howard & Pelc 1953) many of them may also be nuclei in the premitotic G₂-phase, having finished their DNA-synthesis and waiting for diploid mitosis. This possibility deserves consideration in view of the recent observations in many organs that cells may remain in the G₂-phase for several months (Gelfant 1966; Pedersen & Gelfant 1970). Therefore, the term HDC-nuclei (High DNA-quantity Class nuclei) was introduced for nuclei forming separate DNA-content classes with a higher DNA-content than that in the basic class. It was also found in a comparative karyometric and cytophotometric study that the frequency of HDC-nuclei could be indirectly estimated in a satisfactory way by counting nuclei in size classes above the basic class.

HDC-nuclei were found to be more common in toxic goiters than in benign non-toxic goiters without signs of thyroiditis ($p < 0.001$) and in normal thyroids ($p < 0.001$). See fig 4. The frequency of HDC-nuclei in the toxic goiters varied widely, viz from 20-30 % down to a few per cent. Factors found to be positively correlated with the frequency of HDC-nuclei in toxic goiters were lymphoid cell infiltration, recurrence of earlier hyperthyroidism, and demonstrable LATS-activity (fig 5). It was also found (fig 6) that cases, whose hyperthyroid state in the future would recur after thiocarbamide treatment, had higher frequency of HDC-nuclei than those cases which did not recur ($p < 0.01$). As seen in fig 6 the difference was especially apparent when the frequency of HDC-nuclei exceeded 10 %. In the evaluation of the data in fig 6 it should be observed that the four non-relapsing patients with the highest frequency of HDC-nuclei had a cytologically demonstrable lymphoid infiltration in their goiters - a finding connected with a low tendency of hyperthyroidism to recur after thiocarbamide treatment (V). Among those patients who relapsed after long-term thiocarbamide treatment only one had such an infiltration. This patient had a HDC-nuclei percentage of 6.0 and had received a large amount of iodine in cholecystography X-ray contrast just before reappearance of hyperthyroid symptoms - a fact which may have contributed to the relapse (Braverman et al. 1971).

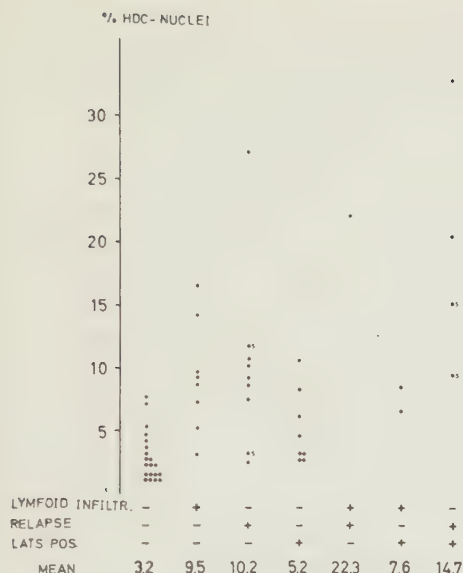


Fig 5. Frequency of HDC-nuclei in different types of toxic goiters. S = relapse after subtotal thyroidectomy. $P < 0.001$ between first and second column as well as between first and third column. $P < 0.05$ for difference between first and fourth column (rank-sum test).

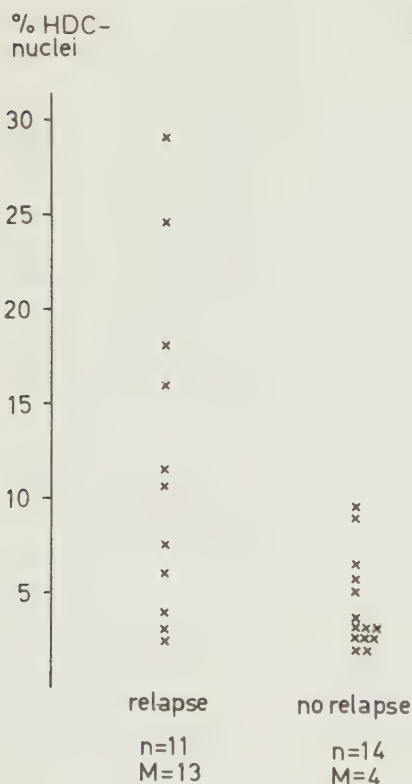


Fig 6. Percentage of HDC-nuclei in cases subsequently relapsing or not relapsing within 18 months of withdrawal of 1-2 years of thiocarbamide treatment. The difference between the two groups was significant with rank-sum test ($p < 0.01$).

Comments

The practical importance of the Jacobj phenomenon described above with regard to the differential diagnosis between toxic and non-toxic goiters is limited. However, large nuclei in the basic class may give reason to suspect an increased hormone production in the follicular epithelium. This can also be said about rich occurrence of HDC-nuclei, but only in the absence of cytological signs of lymphoid thyroiditis. This, because the highest frequency of HDC-nuclei is found in the full-blown cases of the latter condition (unpublished personal observations).

Of great interest was the finding of a particularly strong tendency of hyperthyroidism to recur after long-term thiocarbamide treatment in goiters with HDC-nuclei exceeding 10 % (fig 6). The choice between such treatment and an ablative treatment of thyroid cell mass by surgery or radioiodine is often difficult in thyrotoxicosis. A major disadvantage of the latter type of treatment is the high incidence of subsequent hypothyroidism, which after radioiodine therapy has been estimated to 30-70 % in ten year follow ups (Hagen 1968) and to 3-49 % after surgical therapy (Hedley et al. 1970; Michie et al. 1972). On the other hand, long-term thiocarbamide treatment of 1-2 years is associated with a relapse frequency of about 50 % with most relapses occurring within the first year of withdrawal of the drug (see Hershman et al. 1966). In view of the above-mentioned observations it is natural that studies of factors connected with relapse or remission of hyperthyroidism after thiocarbamide treatment has been a prominent topic in clinical research of thyrotoxicosis. It has thus been stated by several authors that patients prone to relapse have a larger goiter with less tendency to diminish during anti-thyroid treatment as well as longer duration of hyperthyroidism before treatment than those patients which do not relapse (Hershman et al. 1966 and many others). There is, however, a great overlapping between relapsing and non-relapsing cases with regard to the above-mentioned parameters and their practical value is therefore limited in most individual cases. Alexander & Harden (1967) and Hales et al. (1969) claimed that large suppression of 20 minutes ^{131}I -uptake during combined treatment with thiocarbamides and thyroid hormone is a favourable prognostic sign but Lowry et al. (1971) found the difference in such suppressibility between relapsing and non-relapsing cases small and therefore of limited practical value.

The present study of the Jacobj phenomenon (I) introduces a new factor connected with relapse tendency of hyperthyroidism, viz HDC-nuclei. It is noteworthy that all 6 patients with HDC-nuclei above 10 % relapsed, while only 5 of 19 patients with fewer HDC-nuclei relapsed. In the absence

of cytological signs of thyroiditis lesions it therefore seems justified to consider toxic goiters with HDC-nuclei exceeding about 10 %, i.e. cases characterized by a striking discontinuous anisokaryosis as unsuitable for long-term thiocarbamide treatment.

To the workers in routine cytology HDC-nuclei is a phenomenon especially conspicuous in diffuse lymphoid thyroiditis and related to the concept of Askanazy cells, which are common in this disease (Lindsay 1964, Persson 1968). These cells are characterized by large nuclei in addition to certain cytoplasmic traits, such as a peculiar granulation and special staining characteristics (Lindsay 1964, Persson 1968). Thus the definition of HDC-nuclei and Askanazy cells partly coincide. It is interesting to note that the connection between lymphoid cells and HDC-nuclei is demonstrable also in toxic goiters, where the blood level of calorogenic thyroid hormone is high with consequent low blood levels of thyrotropin (Bonnyns et al. 1971) – the latter hormone being known to increase the frequency of HDC-nuclei (Alfert & Kahn 1955). This suggests that a direct influence of lymphoid cells may be of importance for the formation of HDC-nuclei in the follicular epithelium.

HDC-nuclei are sometimes regarded as a sign of cellular proliferation (Garneau 1964, Lindsay 1970). In animal experiments such nuclei have also been found to increase during thyroid stimulation with thiocarbamides and thyrotropin, (Alfert & Kahn 1955; Dobyns & Didschenko 1961), which drugs are known to induce cellular proliferation in the thyroid. However, owing to our poor knowledge of the formation mechanism of such nuclei in human goiters conclusions about the connection between cellular proliferation and HDC-nuclei must be made with caution. Thus, mitotic figures, which are direct signs of proliferation, are extremely rare in untreated toxic goiters even with frequently occurring HDC-nuclei. In an investigation of 97 000 nuclei from the 52 toxic goiters here investigated with regard to HDC-nuclei, only 2 mitotic figures were found (unpublished personal observation).

A HDC-nucleus can only be converted to a non-HDC-nucleus by nuclear division and the low mitotic rate quoted above therefore also suggests that HDC-nuclei are very stable phenomena. Thus, with a mitotic rate of 1:50 000 and an ordinary mitotic duration of 1-2 hours (Cleaver 1967) several months would have to elapse before HDC-nuclei with an initial frequency of, say, 10 % would disappear through nuclear division even if formation of such nuclei was entirely stopped. Therefore the large number of HDC-nuclei by relapse of hyperthyroidism (fig 5) may represent residuals after the preceding toxic episode.

The mode of formation of the HDC-nuclei in toxic goiters is obscure. In analogy with findings in the liver (Alfert & Geschwind 1958; Nadal & Zajdela 1966) such nuclei might be formed by fusion of the nuclei in binucleated cells during a common mitotic phase. In spite of poorly demarcated cell boundaries nuclear constellations in aspirate smears from toxic goiters often suggest the presence of binucleated cells (fig 1). A tendency to cytokinetic failure after nuclear division resulting in formation of such cells is therefore one possible cause of the HDC-nuclei in toxic goiters.

The chromosomal configuration in different types of goiters has been studied by Beierwaltes & Al-Saadi (1966). After 4-10 days of tissue incubation they found that polyploids comprised, on the average, 23 % of the nuclei in mitotic phase in 5 toxic goiters examined. On the other hand, all polyploids in intermitotic phase must by definition be included among the HDC-nuclei, the mean frequency of which was estimated to 7.5 % in toxic goiters in the present investigation. The discrepancy between the above averages suggests that a higher mitotic rate, indicating a stronger proliferation, of polyploid than of diploid cells, may be of importance in the formation of HDC-nuclei in toxic goiters.

MARGINAL VACUOLES (II)

Goiter aspirates have long been studied for characteristics enabling differentiation between toxic and non-toxic goiters. Söderström (1952) found so-called paravacuolar granulations to be more numerous in the cytoplasm of the follicular epithelium in toxic than in non-toxic goiters, but he felt these structures to be of very limited differential diagnostic value (Söderström 1966). The same can be said about the difference in nuclear size between toxic and non-toxic goiters found by Myren & Sivertssen (1962) and corroborated in one part of the present investigation (I). In another part of this investigation (II) the author investigated a peculiar cytoplasmic structure, named marginal vacuoles (MV), supposed to be of some practical value in differentiation between toxic and non-toxic goiters and also thought to be of considerable theoretical interest.

The marginal vacuoles owe their name to their characteristic peripheral

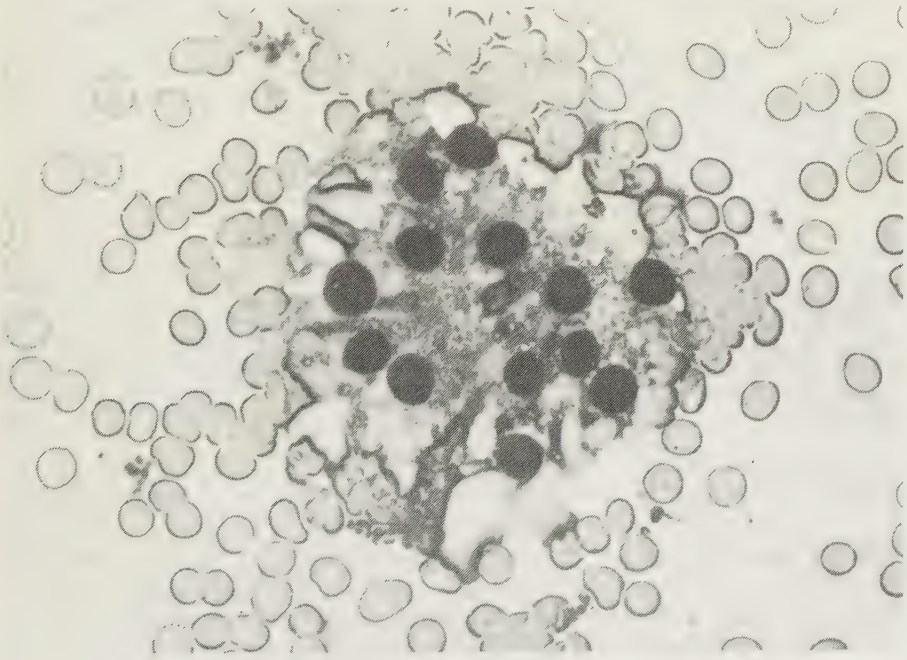


Fig 7. Marginal vacuoles in aspirate smears from toxic goiters. x 160.

position in the cell groups present in aspirate smears (fig 7). With the May-Grünwald-Giemsa staining technique their content usually appears pale with the exception of a faintly red colour of the marginal parts of the vacuoles. The vacuoles are usually of irregular shape and their size varies considerably. Especially toxic goiters may exhibit huge vacuoles larger than the cell nuclei. In such goiters they often constitute a very striking alteration of the follicular epithelium. In the present investigation the marginal vacuoles were not found so distinct in wet-fixed hematoxylin-eosin stained smears as in the May-Grünwald-Giemsa stained smears. They could not be demonstrated with certainty in formalin-fixed histological sections from toxic goiters.

In coded May-Grünwald-Giemsa stained preparations from a series of 49 cases of untreated toxic goiters and 35 cases of benign non-toxic goiters the occurrence of MV was quantitated as follows:

1. None or scanty. Distinct MV exceeding $2\ \mu$ in diameter were demonstrated in no or only a few cells.
2. Moderate. MV were frequently seen, but only in a minority of the cells examined.
3. Abundant. Presence of MV in the majority of the cells examined.

As seen in table 1, MV proved much more common in toxic than in non-toxic goiters ($p < 0.001$). A high frequency of MV thus strongly suggests hyperthyroidism.

Table I

	Abundant MV	Moderate MV	Scanty or none MV
Toxic goiter	21	19	9
Non-toxic goiter	1	10	24

Table I. Occurrence of marginal vacuoles (MV) in different types of fine needle aspirates from the thyroid gland. No cases of cancer or thyroiditis are included in the group of non-toxic goiter. Difference between toxic and non-toxic goiter after pooling of the groups moderate and scanty or none MV: $p < 0.001$.

Comments

The formation mechanism of MC can not be decided with our present scanty knowledge. The difference between their frequency in toxic and non-toxic goiters argues against their being pure artefacts. However, technical factors during the aspiration may influence their appearance in smears. Thus, the very low pressure in the syringe during aspiration (Criborn et al. 1964) may cause expansion of otherwise invisible vacuoles up to a visible size. The author would like to draw attention to one hypothetical ultrastructural counterpart to MV, viz the cisterns of endoplasmic reticulum. In toxic goiters these cisterns may assume a considerable size well within the light microscopical range (Heimann 1966). Their irregular contour also corresponds well to the shape of MV. Their shape, as seen in the microscope, is probably exaggerated by the flattening of cells in air-dried smears. The largest cisterns of endoplasmic reticulum are situated mainly in the basal half of the cells. In dry-fixed smears it is imaginable that the basal parts of the cells tend to be projected free from other cell constituents during flattening of some follicle fragments (fig 8), thereby giving a cytological picture of vacuoles in the marginal parts of the cell groups.

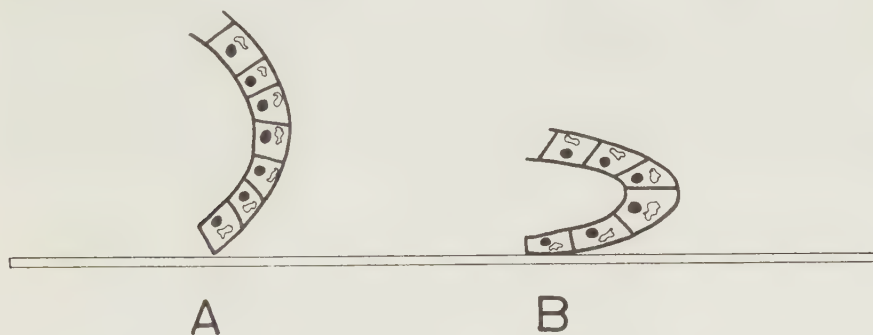


Fig 8. Diagram showing how flattening of a follicle fragment may project the basal part of some cells containing markedly dilated cisterns of endoplasmic reticulum free from other cell constituents.
A: before flattening. B: after flattening.

As seen in table 1, there is considerable overlapping between the occurrence of MV in toxic and non-toxic goiters. However, an abundance of MV strongly suggests the presence of hyperthyroidism and should direct the attention on the possibility of this disease. MV may thereby be of some practical interest in the interpretation of cytodiagnostic fine needle aspirates smears, especially in situations where commonly used laboratory tests are misleading, viz T₃-thyrotoxicosis (Sterling et al. 1970).

During the last decade a new concept has been introduced into microscopical anatomy and physiology of the thyroid gland, viz the parafollicular cells or C-cells, which produce a calcium-lowering polypeptide hormone, calcitonin. In addition to several histochemical characteristics these cells also possess the ability to form certain monoamines (dopamine and serotonin) by decarboxylation of their precursors, which are concentrated in the cytoplasm of the C-cells. Because of their fluorogenic properties these amines as well as their precursors can be detected microscopically by the Falck-Hillarp formaldehyde technique (Falck & Owman 1965).

The clinical interest in C-cells of the human thyroid gland has been especially focused on the neoplastic C-cells transformation in medullary carcinoma (Williams 1970). This tumour contain high levels of catecholamines and 5-hydroxytryptamine (Falck et al. 1968) and is known to store and secrete large quantities of calcitonin into the blood (Cunliffe et al. 1968; Tashjian & Melvin 1968; Milhaud et al. 1968; Meyer & Abdel-Bari 1968; Tashjian et al. 1970; Melvin et al. 1971). However, our knowledge about the microscopical appearance and distribution of C-cells in non-malignant human thyroid is rather meagre (Altman 1940; Sandritter & Klein 1954; Sugiyama 1967; Braunstein & Stephens 1968; Kracht et al. 1968; Kalina et al. 1970), though calcitonin has been demonstrated by bioassay in human goiters (Aliapoulos et al. 1966; Laljee et al. 1967). One major problem in the investigation of such cells in man is that 5-HTP and DOPA, which are especially useful for identification of C-cells because of the fluorogenic properties they possess as well as those of the amines they form, cannot be administered *in vivo* as in laboratory animals. Incubation of goiter biopsy specimens with the above drugs has given poor results (Owman & Sundler: personal communication). However, as shown in III, application of the perfusion technique elaborated by Englund et al. to operative specimens has proved useful for solving this problem. Thus, such a perfusion with L-DOPA in combination with subsequent silver staining produced evidence of the presence of C-cells also in human goiters owing the specific fluorescence of argyrophilic cells evoked under this condition (fig 9).

The identity of the fluorophore in the C-cells demonstrated here is not immediately evident, since not only the administered DOPA, but also



Fig 9. Fluorescence photomicrograph. Central area of thyroid lobe from a case of diffuse toxic goitre after perfusion with L-DOPA. Numerous green-fluorescent cells with a parafollicular localization, x 200. Insert: Higher magnification from the same section to show the cytoplasmic localization of the fluorophore, i.e. the nuclear area is non-fluorescent, x 350.

the corresponding amine, dopamine, gives a formaldehyde-induced fluorescence. Chemical analysis revealed significant amounts of dopamine in the perfused lobed but not in the non-perfused lobe (III). This finding together with the fact that argyrophilic C-cells were the only cells that became fluorescent after L-DOPA administration, provided evidence that at least part of the fluorescence in these cells represents dopamine. Hence, it appears that amine mechanisms operate also in human thyroid C-cells in analogy with findings on C-cells and other polypeptide-producing endocrine cells in several other species (Falck & Owman 1968). One might also imagine that the DOPA-induced dopamine formation and storage in the human C-cells demonstrated here (III) mimics formation of other amines not demonstrable with available histochemical techniques.

The fluorescent C-cells demonstrated in the present investigation were usually found discrete or in small groups of 2-3 cells. Sometimes they were found to give off short irregular processes running close to the follicular epithelium. The number of C-cells varied widely between different goiters and cell counting revealed that they were significantly more numerous ($p = 0.02$) in toxic than in non-toxic goiters. Furthermore their number varied widely from one part of the gland to the other and they were most numerous in the central part of the lobe. They were also present in that part of the lobe which is spared at conventional subtotal thyroidectomy. This finding fits in well with the finding of a greater tolerance to an exogenous calcium load in patients treated with subtotal thyroidectomy than in hyperthyroid patients treated with radioiodine and in patients with spontaneous myxedema (Smith & Laljee 1967, Heydenrich & Bock 1970).

The appearance of C-cells in fine needle aspirates of toxic goiters has to the author's knowledge not been described before. Such aspirates were therefore obtained from the L-DOPA perfused goiters described above. Staining of the smears with May-Grünwald-Giemsa after the formaldehyde treatment necessary for fluorescence studies did not produce especially good results. Thus, cellular details and hues of colours were difficult to distinguish, but in some cases a red cytoplasm and excentric nucleus could be clearly recognized after subsequent staining of green fluorescent cells. The result obtained in the above perfusion studies together with published descriptions of C-cells in histological goiter sections (Altman 1940; Sandritter & Klein 1954) and of malignant C-cells in aspirate smears (Nilsson et al. 1970) allowed distinction of a cell population with C-cells characteristics from the follicular epithelium in aspirate smears from non-malignant goiters (fig 10). These cells often had a faint red cytoplasmic granulation. The

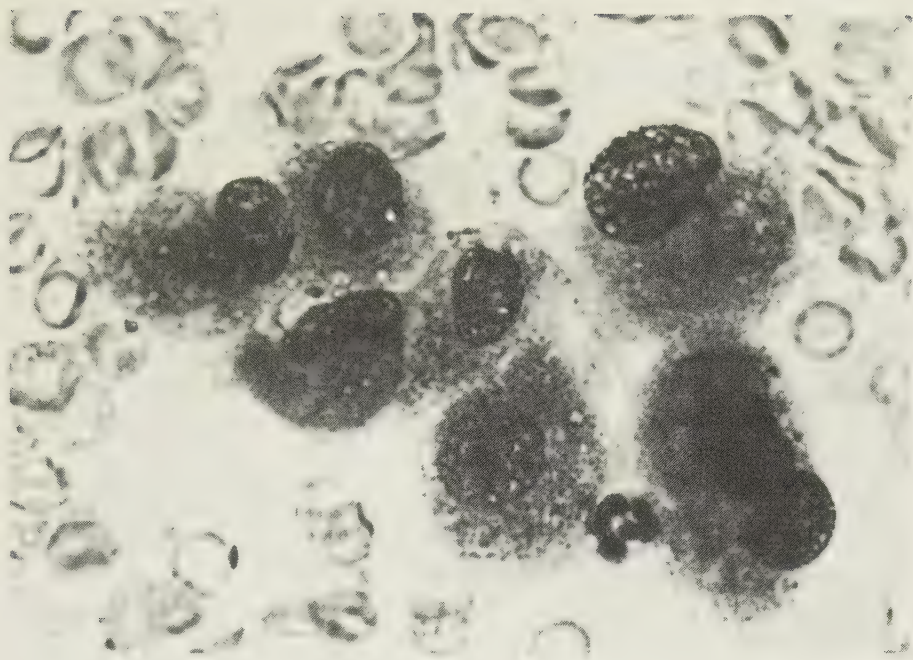


Fig 10. Group of C-cells from a toxic goiter. x 400.

nuclear position was usually eccentric, and bi- or multi-nucleated cells were common. The nuclei were often elongated and pale compared with those of the follicular epithelium. The cytoplasm often appeared irregular and supplied with slender offshoots. An analysis of the occurrence of C-cells so identified in different types of goiters confirmed, that they were more numerous in toxic goiters than in non-toxic goiters ($p < 0.001$). See table 2. Furthermore the frequency of the supposed C-cells decreased with increasing age (fig 11).

Table II

Diagnosis	C-cells demonstrated among 500 follicular epithelial cells		
	> 5	1-5	0
Toxic goiters	31	35	52
Lymphoid thyroiditis	4	5	18
Non-toxic benign goiters without signs of thyroiditis	2	2	26

Table II. Occurrence of C. cells in three types of benign goiter. X^2 diff. biopsies with and without demonstrated C-cells between toxic goiters and lymphoid thyroiditis is 4.13 ($p < 0.05$) and between toxic and non-toxic goiters without thyroiditis 13.3 ($p < 0.001$).

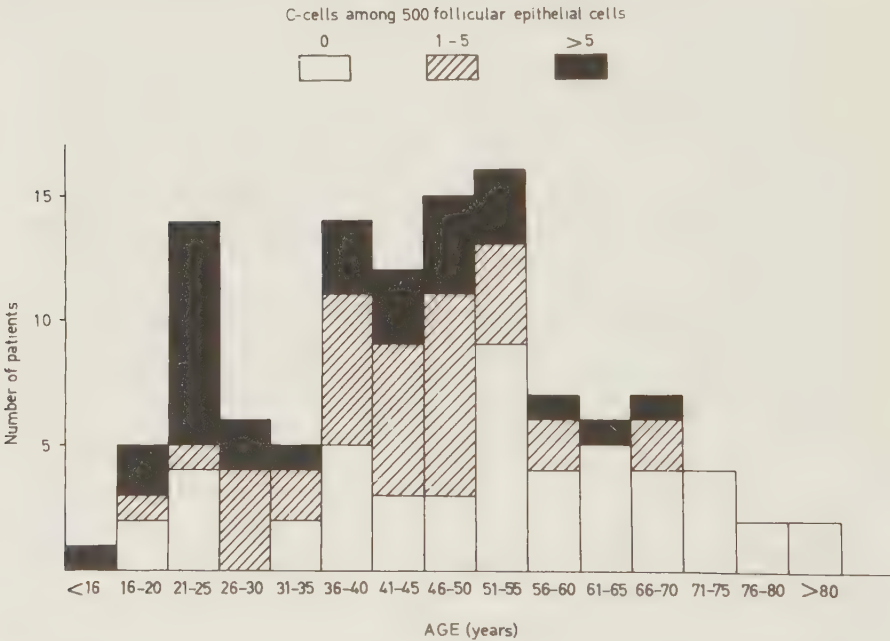


Fig 11. Age distribution by varying degrees of C-cell occurrence in toxic goiters. Mean age for patients with demonstrated C-cells is 40.0 ± 1.7 years, for patients without demonstrated C-cells 52.0 ± 2.5 years (t diff 4.18; $p < 0.001$).

Comments

The occurrence of C-cells in human goiters especially toxic goiters, proved in the present investigation, has many interesting clinical implications. Thus, Melander (1971) found a direct stimulating effect of certain amines, including dopamine, on the hormone secretion of follicular epithelium of rats in vivo. Maayan et al. 1968, 1970, 1971 have obtained similar results in in vitro studies of follicular epithelium. The common occurrence of C-cells provided with offshoots lying close to the follicular epithelium as seen in the 1-DOPA perfused thyroid lobes provides a possible microanatomical basis for a stimulating amine effect on thyroid hormone secretion. In view of the large number of C-cells found in toxic goiters, such an effect may be of importance for the increased hormone production in these goiters.

Bioassay technique have revealed a somewhat lower average calcitonin concentration in toxic than in non-toxic goiters though the difference was not significant (Aliapoulos et al. 1966; Laljee et al. 1967). The discrepancy between these findings and the findings regarding C-cells in the present investigation may, in view of the tendency to hypercalcemia in thyrotoxicosis, be explained in terms of hormone storage. It is thus known from experiments with rats (Gittes et al. 1968) that calcitonin storage is diminished during hypercalcemia. The same mechanism may explain the combination of an especially low concentration of calcitonin (Tashjian & Voelkel 1967) in the thyroid gland and C-cells hyperplasia (Kracht et al. 1968) found during the hypercalcemia induced by hyperparathyroidism. However, recent bioassay of calcitonin in human plasma has failed to demonstrate any differences between euthyroid and hyperthyroid patients (Frazer & Wilson 1971). Obviously a unitarian concept of C-cells and calcitonin secretion in hyperthyroidism is difficult to formulate with the present scanty knowledge. Information about many essential aspects of the problem such as the rate of calcitonin secretion and extent of calcitonin storage in the numerous C-cells of toxic glands is still lacking. Furthermore, it cannot be excluded that the cells equipped with amine-handling mechanisms demonstrated in the present work (III) produce other polypeptides, not yet discovered, in addition to calcitonin. A parallel to such a situation is found in Langerhan's islets of pancreas, where the production of three very potent polypeptides, viz insulin, glucagon, and gastrin has been traced to cells equipped with the same amine-handling mechanisms as those here demonstrated in human C-cells.

LYMPHOID INFILTRATION OF TOXIC GOITERS (V)

Lymphoid infiltration of toxic goiters has long been recognized from histological examination of surgical specimens (Brünger 1915). This infiltration is usually mild, but in some toxic goiters lymphoid tissue may constitute more than half of the goiter volume (Whitesell 1949). The lymphoid tissue is most often represented by small foci with a cellular composition identical with that found in fullblown Hashimoto goiters (Harris 1969). Furthermore, microscopically the lymphoid cell infiltrate found in both toxic goiters and Hashimoto goiters resembles that found in autoimmune experimental thyroiditis in animals (Backwinkel et al. 1970) as well as in other conditions thought to be due to delayed hypersensitivity, such as allografts undergoing rejection (Porter et al. 1964).

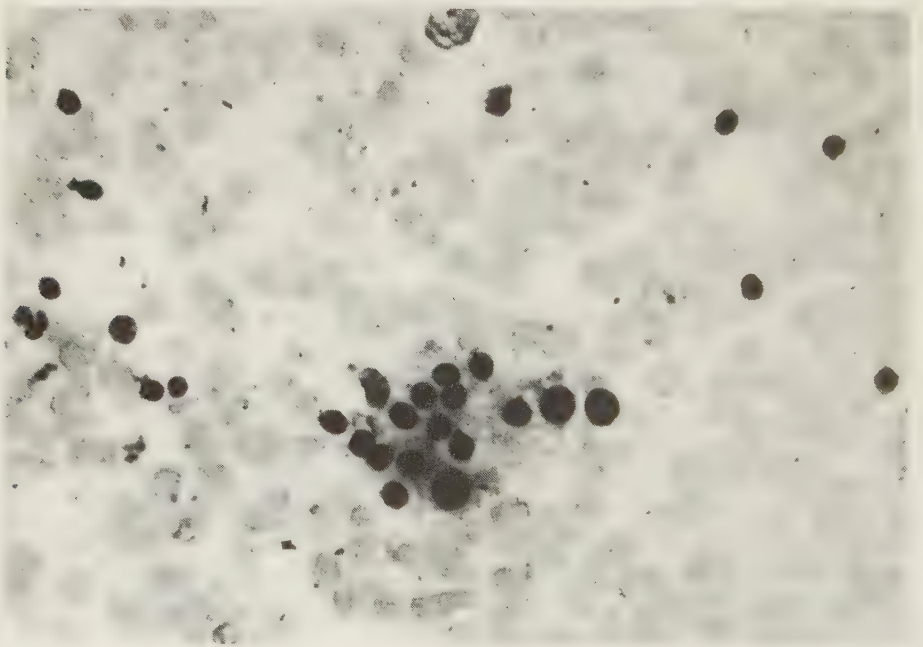


Fig 12. Smears prepared from fine needle aspiration biopsy specimens of toxic goiter showing a group of follicular epithelium among numerous lymphoid cells. 40 x.

The lymphoid tissue in toxic goiters seems to be part of a generalized hyperplasia of lymphoid tissue (Warthin 1928; Wolf 1937) including the thymus (Michie et al. 1967) in which lymphoid follicles are much more common in combination with toxic than with non-toxic goiters (Gunn et al. 1964). Furthermore, in hyperthyroidism thymic lymphoid follicles are particularly often found in the presence of lymphoid follicles in the goiter (Gunn et al. 1964).

As expected from the above findings, some fine needle aspirates from toxic goiters revealed an excess of lymphoid cells, which were usually evenly distributed over the smear (fig 12) suggesting that the lymphoid foci had been broken up during aspiration. Comparison with the number of lymphoid foci in available surgical specimens indicated that an excess of lymphoid cells in aspirate smears corresponded to the histological sections with most numerous lymphoid foci (fig 13). In some cases with a considerable number of lymphoid foci, however, this condition was not detected in the aspirate smears (fig 13) probably because of focal distri-

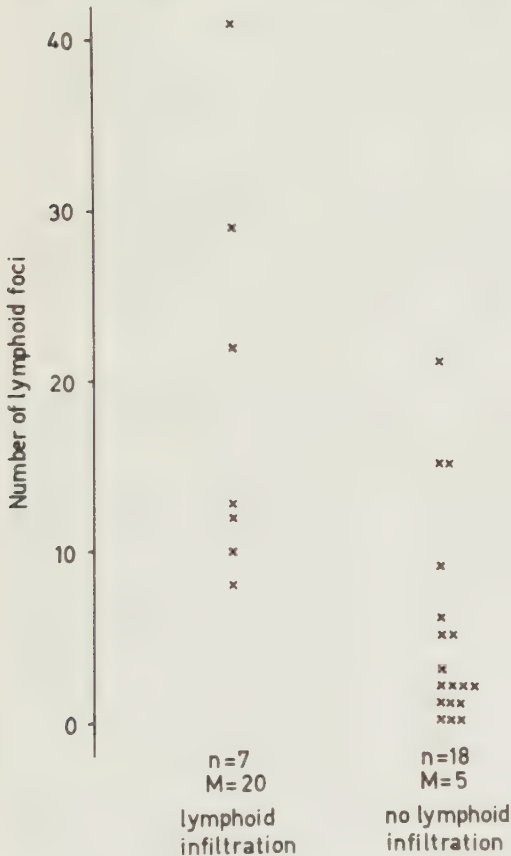


Fig 13. Number of lymphoid foci in 50 fields of vision (x 12.5) in histological sections of toxic goiters with and without cytologically demonstrable infiltration of lymphoid cells. Rank-sum test showed a significant difference between the two groups ($p < 0.001$).

bution of the lymphoid foci or to large an admixture of blood to the fine needle aspirates. It must also be borne in mind that the surgical specimens aspirates. It must also be borne in mind that the surgical specimens and the fine needle aspirates are not quite comparable because of the preoperative antithyroid treatment with carbimazol and l-thyroxin. Thus, it is known that thymic lymphoid tissue markedly diminishes in size during preoperative treatment with thiocarbamides (Michie et al. 1967). Such a reduction of the lymphoid tissue may also occur in the thyroid itself.

By comparison of clinical data and lymphoid infiltration in toxic goiters as evidenced by fine needle aspirates some conclusions of interest could be made:

1) There was a correlation between lymphoid infiltration and the occurrence of circulating thyroid antibodies against thyroglobulin and/or cytoplasmic antigen ($p < 0.05$). When the lymphoid infiltration was correlated with only antibodies against thyroglobulin or with cytoplasmic thyroid antibodies the correlation was not certain ($0.10 > p > 0.05$). These results agree with the correlation found between lymphoid infiltration of the thyroid, as assessed histologically, and circulating thyroid autoantibodies (Roitt & Doniach 1958; Schade et al. 1960; Irvine et al. 1962; Hargreaves & Garner 1968). However, in this connection it is noteworthy that investigations in autoimmune experimental thyroiditis have shown that skin tests, which reflect delayed hypersensitivity, have much closer association with thyroiditic lesions than the circulating autoantibodies (Mc Master et al. 1961; Miescher et al. 1961; Rose et al. 1962; Jancovic & Mitrovic 1963). These results indicate a central role of cellular immunity in the pathogenesis of thyroiditic lesions. This is also supported by demonstration of lymphoid cell cytotoxicity against follicular epithelium in rats with experimental thyroiditis (Biörklund 1968) as well as in Hashimoto's disease (Laryea et al. 1971).

One notable finding in the present investigation was the lack of correlation between LATS and lymphoid infiltration of the thyroid. Most investigations of the connection between thyroid autoantibodies and LATS have failed to show any connection between these two types of immunoglobulins (Pinchera et al. 1967; Beall & Solomon 1968; Fagreus et al. 1970) though one conflicting report claims that there is a positive correlation between LATS and antithyroglobulin antibodies (Bastenie et al. 1967). Most evidence therefore points to LATS as a phenomenon not related to other serological and morphological signs of thyroid autoimmunity.

2) Lymphoid infiltration in toxic goiters revealed a peculiar age distribution (fig 14) and was more seldom found in patients above than below 55 years ($p < 0.02$). Furthermore, the hyperthyroid patients with lymphoid

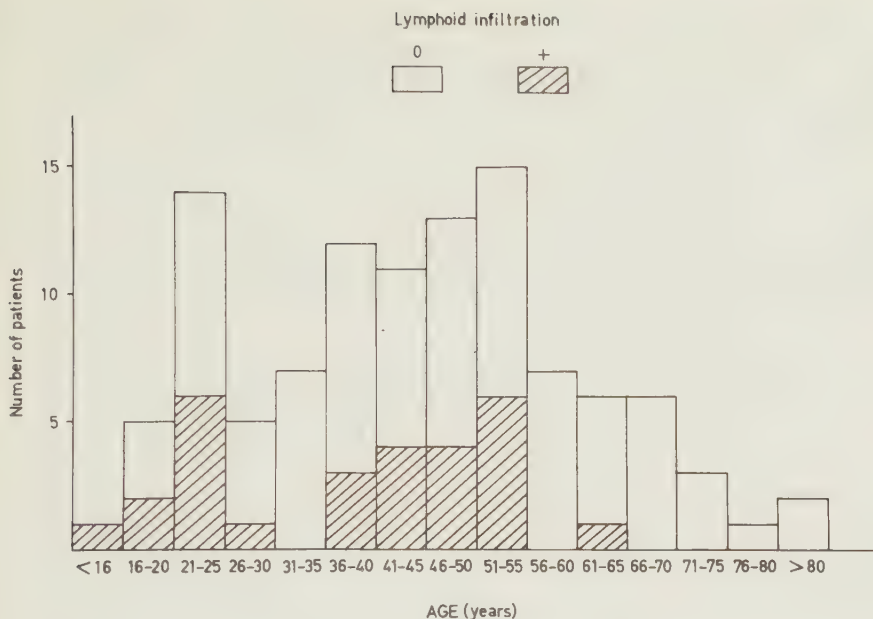


Fig 14. Age distribution of patients examined regarding cytologically demonstrable lymphoid infiltration in their goiters. Hatched areas denote patients with such infiltration. The mean in the latter group was 38.5 ± 2.7 and for the remaining patients 46.6 ± 1.8 . The difference was significant ($t = 2.31$; $p < 0.025$).

infiltration in their goiters tended to crowd around 20-25 and 40-50 years of age thereby strongly contributing to give the age distribution of the whole series of toxic goiter patients a double-peaked shape. The low incidence of lymphoid infiltration in higher ages may be an expression of general decline of lymphoid tissues in these ages. Thus it has been found that the spleen weight decreases after 50 years (Hellman 1926). In patients with thyrotoxicosis as well as those with non-toxic nodular goiters the volume of lymphoid tissue in thymus decreased with increasing age (Michie et al. 1967).

The double-peaked age distribution of toxic goiters and especially those toxic goiters with lymphoid infiltration found in the present series is peculiar. It does not agree with the age distribution found in many series of toxic goiters (Iversen 1948; Heilmann 1962; Lamberg 1969) and may be an insignificant finding. However, the author has observed a recent series

of hyperthyroid patients with quite a similar age distribution (Hjort et al. 1970). Furthermore the occurrence of thyroid autoantibodies, which, as pointed out earlier is a phenomenon related to lymphoid infiltration, has been found to be especially marked around 20 years and 40-50 years (Evans et al. 1967; Hjort et al. 1970). The same was found for the relatives of the hyperthyroid patients (Evans et al. 1967). A notable finding pointed out by Michie et al. (1971) is the especially high incidence of post-operative hypocalcemia in thyrotoxic patients about 20-30 and 40-50 years age. Thus, there is accumulating evidence of differences in the nature of the thyrotoxic disease at different age levels.

3) The present investigation provided evidence of a relationship between demonstrable lymphoid infiltration in fine needle aspirates and the results of long-term thiocarbamide treatment. As pointed out earlier in the discussion about HDC-nuclei investigation of several large patient series (see Hershman et al. 1966) has shown that there is a relapse frequency of about 50 % after such treatment with most of the relapses occurring within the first year of withdrawal of the drug. Some factor such as large goiter size, long duration of symptoms, and marked T_3 suppression of 20 minutes radioiodine uptake by the thyroid have been connected with a high relapse rate (Hershman et al. 1966; Alexander & Harden 1967; Hales et al. 1969; Lowry et al. 1971). The overlap in individual values between cases of recurring and non-recurring hyperthyroidism, however, makes the estimation of the risk of recurrence after thiocarbamide treatment very difficult. The present investigation provide evidence that cases with demonstrable lymphoid infiltration in goiter aspirates are suitable for long-term treatment with thiocarbamides. Thus hyperthyroidism recurred in only one of nine patients with lymphoid infiltration during the first eighteen months after withdrawal of the drug. It is noteworthy that the relapsing patient recieved a large amount iodine in cholecystography X-ray contrast just before reappearance of hyperthyroid symptoms. This may have contributed to the relapse (Braverman et al. 1971). Irvine (1967) failed to find a similar prognostic significance for thyroid antibodies, as here found for lymphoid infiltration. However, in view of the earlier mentioned central role of cellular immunity in thyroiditic lesions it seems reasonable to assume that lymphoid cells within the thyroid are more essential for the development of thyroid function than the conventionally used serological tests for thyroid autoantibodies.

GENERAL DISCUSSION

The present investigation was performed at a clinic where fine needle biopsies have been extensively used in the examination of different types of goiters - also toxic ones. This practice has been adapted because the method is simple and safe and often yields valuable and unexpected diagnostic findings, such as malignant and thyroiditic lesions. The present paper is the first record of the author's experience with goiter cytology with thyrotoxicosis as a common denominator.

One reason for fine needle aspirations also of toxic goiters is the not uncommon occurrence of carcinomas in such goiters. In a compilation of several large series Sokal (1954) found hyperthyroidism in 9.6 % of all patients with thyroid cancer and in a recent Finnish series of thyroid cancer the corresponding figure was 11 % (Sivula 1969). The incidence of carcinoma in comprehensive compilations has been estimated at 0.94 % in toxic nodular goiters and 0.15 % in toxic diffuse goiters (Sokal 1954). A still higher incidence, 2.5 and 3.8 % respectively of cancer in toxic goiters has recently been claimed by other authors (Olen & Klinck 1966; Georgiadis et al. 1971). Most of the malignant lesions in these series were small papillary carcinomas but anaplastic carcinomas were also found. No distant metastases were observed, but lymphoglandular and intrathyroidal metastases were noted. No malignant lesions were found in the present series of cytologically examined toxic goiters. The value of cytodiagnostic fine needle aspiration biopsies in diagnosing malignant lesions in the thyroid has however been pointed out earlier (Einhorn & Franzén 1962; Söderström 1966) and has been confirmed also in this laboratory.

In addition to the question of malignancy it was considered essential to focus interest on three questions, delineated in the introduction of this thesis, by examining aspirate smears from toxic goiters. The first question concerns the value of goiter aspirates in distinguishing toxic and non-toxic goiters. To the author's disappointment no clear-cut differentiation between the two types of goiters could be obtained by the cytological approach tried in the present investigation. It was evident, however, that abundant marginal vacuoles are much more common in toxic than in non-toxic goiters. Consequently, a rich occurrence of these structures ought to direct the examiner's attention to the possibility of

hyperthyroidism. It is also noteworthy that, compared with non-toxic goiters, the cell nuclei in toxic goiters are larger with a larger proportion of cells in separate DNA-content classes (HDC-nuclei).

The second question concerns the prognostic significance of different cytological features with regard to relapse of hyperthyroidism after long-term thiocarbamide treatment. The relapse rate after such treatment has been estimated at about 50 % in several large series (see Hershman et al. 1966) with most of the relapses occurring within the first year after withdrawal of the drug. On the other side ablative treatment of toxic goiters by surgery or radioiodine is connected with important disadvantages such as an unsatisfactorily high rate of posttherapeutic hypothyroidism (Hagen 1968; Hedley et al. 1970; Michie et al. 1972). In view of these facts prediction of hyperthyroidism recurrence after long-term thiocarbamide treatment is of large importance in the choice of the most adequate treatment for a given patient. Goiter size, duration of symptoms, and suppression of 20 minutes radioiodine uptake (Hershman et al. 1966; Alexander & Harden 1967; Hales et al. 1969; Lowry et al. 1971) have proved to be of some predictive value but in most individual cases prediction of recurrence after thiocarbamide treatment is most uncertain. The present investigation suggests that lymphoid infiltration of the goiter, as judged from examination of aspirate smears, is associated with a substantially lower recurrence rate than in cases without such infiltration. The presence of lymphoid infiltration therefore provides an argument for thiocarbamide treatment, particularly as surgical removal of the goiter in such a situation is known to be associated with an especially high rate of hypothyroidism (Whitsell et al. 1949; Greene 1950; Lewitt 1951; Hargreaves et al. 1958). It was also found that an abundance of cells in size classes above the basic class, which have been shown to correspond to cells in DNA-content classes above the basic class (= HDC-nuclei) is connected with high recurrence tendency after such treatment. Thus long-term thiocarbamide treatment of goiters with HDC-nuclei above 10 %, i.e. cases with a striking discontinuous anisokaryosis, seem unsuitable in the absence of lymphoid infiltration.

The third question formulated for the present investigation concerned cytological findings of possible pathogenetic interest with regard to thyrotoxicosis. There is no generally accepted theory of the mechanism of this disease. Thus the explanation for the hyperthyroidism in Grave's disease offered some years ago by LATS, seems doubtful in view of recent investigations (Chopra et al. 1970 a, b). On the other hand, other substances have recently been found to be able to influence the function of the follicular epithelium. Thus Mayaan et al. (1968, 1970, 1971) in

in vitro studies and Melander (1971) in in vivo studies have produced clear evidence that catecholamines and serotonin augment the hormone secretion of the follicular epithelium. In this connection it is interesting to note the finding, in the present investigation, of abundant C-cells capable of forming such amines. It is therefore tempting to assume a possible role of amines in the pathogenesis of thyrotoxicosis, although our present knowledge does not permit any conclusions in this field.

Another interesting mechanism for the stimulation of the follicular epithelium has recently been demonstrated by Edmonds et al. (1970). These authors found that lymphocytes from peripheral blood of patients with Grave's disease can stimulate the function of the follicular epithelium. This stimulation occurred even in the absence of LATS-activity in the blood. The possibility of a pathogenetic role of lymphocytes in Grave's disease has also been suspected by the curative effect of cortisone demonstrated in this disease (Werner et al. 1965). The above findings witness the possible pathogenetic implications of the lymphoid infiltration of toxic goiters earlier demonstrated (Brünger 1915 and many others) and confirmed also in the present investigations. Fine needle aspiration often provide interesting aspects of the connection between lymphocytes and follicular epithelium. For instance, a close connection between the two cell types is often beautifully illustrated in the constellations of cells in aspirate smears, where the lymphocytes is found in very intimate contact with the cytoplasm of the epithelial cells, suggesting the occurrence of an emperipoles mechanism (Humble et al. 1956).

GENERAL SUMMARY AND CONCLUSIONS

The experienced cytologist may recognize or at least suspect thyrotoxicosis from the cytologic picture of a fine needle goiter aspirate. The purpose of the present investigation was to define cytomorphologic features allowing these conclusions. Particular interest was focused also on the relationship between cytological findings and the tendency of hyperthyroidism to recur after long-term thiocarbamide treatment. This was done to define the possible role of fine needle aspirates in the important selection between long-term thiocarbamide treatment of the toxic goiter and ablation of the goiter by surgery or radioiodine. Furthermore, in the course of the investigation some features of fine needle aspirates of possible pathogenetic interest with regard to thyrotoxicosis were noted and analysed in some detail.

The following results were obtained.

Nuclear size

Previous observations that the nuclear diameter of the follicular epithelium is larger in toxic than in non-toxic goiter was confirmed also in this investigation. In addition, it was noted that nuclear size variation in aspirate smears from toxic goiter was often characterized by the existence of distinctly separate nuclear size classes, which give the examining cytologist a strong impression of a discontinuous nuclear size variation. Thus, most nuclei formed one size class, in which the average diameter was significantly larger in toxic than in non-toxic goiters. A minor number of large nuclei, whose DNA-content was approximately two or four times that of the basic class, formed one or two separate size classes. The latter nuclei were called HDC-nuclei (= High DNA-content Class nuclei). They were found to be significantly more common in toxic than in non-toxic goiters. In toxic goiter they were significantly correlated with relapse of earlier hyperthyroidism, cytologically demonstrable lymphoid infiltration, and presence of long acting thyroid stimulating factor, LATS, in serum. It was also found that cases, whose hyperthyroid state in the future would recur after

thiocarbamide treatment, had a significantly higher frequency of HDC-nuclei than those cases which did not recur after such treatment. This difference was especially apparent if cases with lymphoid infiltration were excluded. It was thus possible to distinguish a subgroup of toxic goiters without lymphoid infiltration and with HDC-nuclei exceeding about 10 %, giving rise to an striking discontinuous nuclear size variation in the aspirate smears. This subgroup, which comprised 6 of 25 goiters in the present material, seems unsuitable for long-term thiocarbamide treatment because of a high rate of posttherapeutic relapse. In view of the probably extremely low turnover HDC-nuclei this finding is in good agreement with the above-mentioned observation of a high percentage of HDC-nuclei in toxic goiters with relapse of earlier hyperthyroidism.

Marginal vacuoles

A curious type of vacuoles in the cytoplasm of the follicular epithelium, here called marginal vacuoles (MV), was found to be much more common in toxic than in non-toxic goiters. If these structures are abundant they seem to merit serious attention as signs of hyperthyroidism, as judged from the present investigation. It is impossible to decide the formation mechanism of MV with our present knowledge but the author found some reasons to suggest dilated cisterns of the endoplasmic reticulum as a possible ultrastructural counterpart to these structures.

C-cells

In aspirate smears from toxic goiters the author early observed a specific type of cells, which appeared closely related to the cells observed in aspirate smears from medullary carcinoma - a tumor thought to originate from the parafollicular C-cells. The identity of the above-mentioned cells in toxic goiter aspirates with C-cells was secured by ex vivo L-DOPA perfusion of resected human goiters. Thereby the C-cells revealed a specific fluorescence by use of the Falck-Hillarp formaldehyde technique. The C-cells proved identifiable in aspirate smears and were found to be more common in toxic than in non-toxic goiters - a finding which was in good agreement with the observations in histological sections of the L-DOPA perfused goiters. Though these findings have little importance in practical diagnosis they are of interest with regard to the pathophysiology of thyrotoxicosis. The hypothesis was put forward that C-cells may sti-

mulate the function of the follicular epithelium through local release of certain amines, which recently in animal experiments have proved to increase secretion of thyroid calorigenic hormone.

Thyroiditic lesions

A close connection was noted between the number of lymphoid foci in histological sections from surgical specimens of toxic goiters and increased number of lymphoid cell in aspirate smears from the same goiters. However, possibly owing to a large admixture of blood and focal distribution of the thyroiditic lesions some cases with a considerable number of lymphoid foci in the sections were not detected in the aspirate smears. It must thus be emphasized that a fine needle aspirate does not exclude thyroiditic lesions though a positive diagnosis of such a lesion could be obtained with this method. In a series of aspirate smears from toxic goiters increased number of lymphoid cells was found to be positively correlated with the occurrence of antibodies against thyroglobulin and/or cytoplasmic antigen, but not with the long-acting thyroid stimulating factor, LATS. Lymphoid cell excess was seldom found in elderly patients. When present it was found to be connected with a substantially low relapse rate of hyperthyroidism after long-term thiocarbamide treatment. This made it possible to conclude that cytological evidence of lymphoid cell excess in toxic goiters is a positive argument for long-term thiocarbamide treatment. Furthermore, surgical ablation of toxic goiters with thyroiditic lesions is not an attractive alternative because of an especially high rate of postoperative hypothyroidism found in many investigations.

REFERENCES

- ALEXANDER, W.D. & HARDEN, R.: In Thyrotoxicosis, Proceedings of an Internal. Symp. (ed W.J. Irvine)p 99, Edinburgh 1967.
- ALFERT, M.H.A. & KAHN, R.H.: Acta anat. 23:185, 1955.
- ALFERT, M.H.A. & GESCHWIND, I.I.: Exp. Cell Res. 15:230, 1958.
- ALIAPOULIOS, M.A., VOELKEL, E.^F & MANSON, P.L.: J. Clin. Endocr. 26:897, 1966.
- ALTMAN, H.W.: Beitr. Path. Anat. 104:420, 1940.
- BACKWINKEL, K.P., SCHADE, H. & THEMANN, H.: Beitr. path. anat. 140:362, 1970.
- BASTENIE, P.A., BONNYNS, M. & VANHAELST, L.: In Thyrotoxicosis, Proceedings of an Internat. Symp. (ed W.J. Irvine) p. 40. Edinburgh 1967.
- BEALL, G.N. & SOLOMON, D.H.: Clin. Exp. Immunol. 3:615, 1968.
- BEIERWALTES, W.H. & AL-SAAD, A.A.: J. Clin. Endocr. 26:729, 1966.
- BIÖRKLUND, A.: Studies on experimental autoimmune thyroiditis in the rat. Studentlitteratur, Lund 1968.
- BONNYNS, M., VANHAELST, L., DELESPESE, G., BASTENIE, P.A. & ERMANS, A.M.: In Thyroiditis and Thyroid Function (eds Bastenie, P.A. & Ermans, A.M.) p. 171. Pergamon Press 1972.
- BRAUNSTEIN, H. & STEPHENS, C.L.: Arch. Path. 86:659, 1968.
- BRAVERMAN, L., VAGENAKIS, A., WANG, C., MALOOF, F. & INGBAR, S.: Paper read at the 4th Meeting of the European Thyroid Association. Berne 1971.
- BRÜNGER, H.: Mitt a.d. Grenzgeb. d. Med. Chir. 28:213, 1915.
- BURSTONE, M.S.: J. Nat. Cancer Inst. 21:523, 1958.
- CASPERSSON, T. & LOMAKKA, G.: Ann. N.Y. Acad. Sci. 97:449, 1962.
- CHOPRA, I.J. & SOLOMON, D.H.: Ann. Int. Med. 73:985, 1970.
- CHOPRA, I.J., SOLOMON, D.H., JOHNSSON, D.E. & CHOPRA, U.: Metabolism 19:760, 1970.
- CLEAVER, J.E.: In Thymidine Metabolism and Cell Kinetics (eds Neuberger, A & Tatum, E.L.) p. 125. North Holland Publishing Co Amsterdam 1967.
- CASPERSSON, T. & LOMAKKA, G.: Ann. N.Y. Acad. Sci. 97:449, 1962.
- CRIBORN, C.O., FRANZÉN, S., UNSGAARD, B. & ZAJICEK, J.: Scand. J. Haemat. 1:272, 1964.
- CUNLIFFE, W.J., BLACK, M.M., HALL, R., JOHNSTON, I.D.A., HUDGSON, P., SHUSTER, S., GUDMUNDSSON, T.W., JOPLIN,

- G. F., WILLIAMS, E. D., WOODHOUSE, N. J. Y., GALANTE, L. & MacINTYRE, I., *Lancet* II: 63, 1968.
- DOBYNS, B. M. & DIDTSCHENKO, I.: *J. Clin. Endocr.* 21:699, 1961.
- EDMONDS, M. E., ROW, V. V. & VOLPE, R.: *J. Clin. Endocr.* 31:480, 1970.
- EINHORN, J. & FRANZEN, S.: *Acta Radiol.* 58:321, 1962.
- ENGLUND, N. E. & VANG, J. To be published.
- EVANS, A. W. H., McDOUGALL, C. D. M., WOODROW, J. G. & CHEW, A. R.: *Lancet* I:636, 1967.
- FAGREUS, A., JONSSON, J. & EL KABIR, D.: *J. Clin. Endocr.* 31:445, 1970.
- FALCK, B. & OWMAN, Ch.: *Acta Univ. Lund* II 7:1, 1965.
- FALCK, B. & OWMAN, C.: *Adv. Pharmac.* 6:211, 1968.
- FALCK, B., LJUNGBERG, O. & ROSENGREN, E.: *Acta Path. microbiol. scand.* 74:1, 1968.
- FRAZER, S. & WILSON, G. M.: *Lancet* I:725, 1971.
- GARNEAU, R.: *Laval Medical* 35:188, 1964.
- GELFANT, S.: *Methods in Cell Physiology* (ed D. M. Prescott) vol. 2, p. 359. AP New York 1966.
- GEORGIADIS, N. J., LEONTSAKOS, B. G. & KATSAS, A. G.: *Internat. Surg.* 55:27, 1971.
- GITTES, R. G., TOVERUD, S. U. & COOPER, C. W.: *Endocrinology* 82:83, 1968.
- GREENE, R.: *J. Clin. Endocr.* 7:1, 1950.
- GUNN, A., MICHIE, W. D. & IRVINE, W. J.: *Lancet* 2:776, 1964.
- HAGEN, G. A.: *Med. Clin. North Am.* 52:417, 1968.
- HALE, A. J.: *J. Path. Bact.* 85:311, 1963.
- HALES, I., STIEL, J., REEVE, T., HEAP, T. & MYHILL, J.: *J. Clin. Endocr.* 29:998, 1969.
- HARGREAVES, W. & GARNER, A.: *Brit. J. Surg.* 55:543, 1958.
- HARRIS, H.: *J. Cell Sci.* 2:23, 1967.
- HARRIS, M.: *J. Clin. Path.* 22:326, 1969.
- HEDLEY, A. J., FLEMING, C. J., CHESTERS, M. J., MICHIE, W. & CROOK, J.: *Br. M. J.* 1:519, 1970.
- HEIMANN, P.: *Acta Chir. Scand. Suppl.* 289, 1962.
- HEIMANN, P.: *Acta Endocr. Suppl.* 110, 1966.
- HELLMAN, T.: *Z. Vererb. u. Konstit. Lehre.* 12:270, 1926.
- HERSHMAN, J. M., GIVENS, J. R., CASSIDY, C. E. & ASTWOOD, E. B.: *J. Clin. Endocr.* 26:803, 1966.
- HEYDENRICH, G. & BOCK, J. E.: *Nordisk Medicin* 83:238, 1970.
- HJORT, T., LAURIDSEN, U. B. & PERSSON, I.: *Acta Med. Scand.* 188:431, 1970.
- HOWARD, A. & PELC, S. R.: *Heredity. Suppl.* 6:261, 1953.
- HUMBLE, J. G., JAYNE, W. H. W. & PULVERTAFT, R. J. W.: *Br. J. Haemat.* 2:283, 1956.

- IRVINE, W. J., MCGREGOR, A. G. & STUART, A. E.: *Lancet* 2:843, 1962.
- IRVINE, W. J., & STEWART, E. G.: In *Thyrotoxicosis. Proceeding of an Internat. Symp.* (ed. W. J. Irvine) p. 111. Edinburgh 1967.
- IVERSEN, K.: *Temporary rise in the Frequency of thyrotoxicosis in Denmark 1941-45.* Rosenkilde & Bagger, Copenhagen 1948.
- JACOBJ, W.: *Arch. Entw. Mech. Org.* 106:124, 1925.
- JACOBJ, W.: *Ztschr. f. mikr. anat. Forsch.* 38:161, 1935.
- JANCOVIĆ, B. D. & MITROVIĆ, K.: *Nature* 200:186, 1963.
- KALINA, M., FOSTER, G. W., CLARK, M. B. & PEARSE, A. G. E. in: *Calcitonin 1969. Proc. 2nd. Internat. Symp.* (ed. S. Taylor) p. 268. Heinemann, London 1970.
- KRACHT, J., HACHMEISTER, U., KRUSE, H. & MATTHAES, P.: *Verh. Dtsch. Ges. Path.* 52:485, 1968.
- LALJEE, H. C. K., SMITH, R. W. & DORRINGTON, K. J.: *J. Endocr.* 39:507, 1967.
- LAMBERG, B. A.: *Sköldkörtelnns sjukdomar* p. 132. Helsingfors 1969.
- LARYEA, E., ROW, V. V. & VOLPÉ, R.: Paper read at the 4th Meeting of the European Thyroid Association, Berne 1971.
- LEWITT, T.: *Lancet* II:957, 1951.
- LINDSAY, S.: In *The Thyroid gland vol. 2* (eds. Pitt-Rivers & Trotter) p. 223. Butterworths London 1964.
- LINDSAY, S.: *Surg. Gyn. & Obst.* 131:905, 1970.
- LOWRY, R. C., LOWE, D., HADDEN, D. R., MONTGOMERY, D. A. D. & WEAVER, J. A.: *Br. M. J.* 2:19, 1971.
- MAAYAN, M. L. & INGBAR, S. H.: *Science* 162:124, 1968.
- MAAYAN, M. L. & INGBAR, S. H.: *Endocrinology* 87:588, 1970.
- MAAYAN, M. L., MILLER, S. L. & INGBAR, S. H.: *Endocrinology* 88:620, 1971.
- McKENZIE, J. M.: *Endocrinology* 63:372, 1958.
- Mc MASTER, P. R. B., LERNER, E. M. & EXUM, E. D.: *J. exp. Med.* 113:611, 1961.
- MELANDER, A.: *Acta phys. scand. Suppl.* 307, 1971.
- MELVIN, K. E. W., MILLER, H. H. & TASHJIAN, A. A.: *N. Engl. J. Med.* 285:1115, 1971.
- MEYER, J. S. & ABDEL-BARI, W.: *New Engl. J. Med.* 278:530, 1968.
- MICHIE, W., BECK, Y. S., MAHAFFY, R. G., HONEIN, E. F. & FOWLER, G. B.: *Lancet* 1:691, 1967.
- MICHIE, W., STOWERS, J. M., DUNCAN, T., PEGG, C. A. S., HAMER-HODGES, D. W., HEMS, G., BEWSHER, P. D. & HEDLEY, A. J.: *Lancet* 1:508, 1971.
- MICHIE, W., PEGG, C. A. S. & BEWSHER, P. D.: *Br. M. J.* 1:13, 1972.
- MIESCHER, P., GORSTEIN, F., BENACERAFF, B. & GILL, P. G. H.: *Proc. Soc. exp. Biol.* 107:12, 1961.
- MILHAUD, G., TUBIANA, M., PARMENTIER, C. & CONTRIS, G.: *C.R. Acad. Sci. Paris* 266:608, 1968.

- MYREN, J. & SIVERTSSEN, E.: *Acta Endocr.* 39:431, 1962.
- NADAL, C. & ZAJDELA, F.: *Exp. Cell Res.* 42:99, 1966.
- NILSSON, G., SÖDERSTRÖM, N. & TELENIOUS, M.: *Lancet* II:666, 1970.
- NOSSLIN, B.: *Scand. J. Clin. Lab. Invest. Suppl.* 86:177, 1965.
- OEHLERT, W. & SCHULTZE, B.: *Beitr. pathol. Anat.* 123:101, 1960.
- OLEN, E. & KLINCK, G.H.: *Arch. Path.* 81:531, 1966.
- PEDERSEN, T. & GELFANT, S.: *Exp. Cell Res.* 59:32, 1970.
- PERSSON, S.: *Acta Med. Scand. Suppl.* 483, 1968.
- PINCHERA, A., LIBERTI, P., de SANTIS, R., GRASSO, L., MARTINO, E. & BASCHIORI, L.: *J. Clin. Endocr.* 27:1758, 1967.
- PORTER, K.A., JOSEPH, N.H., RENDALL, J.M., STOLINSKI, C., HOEHN, R.J. & CALNE, R.Y., *Lab. Invest.* 13:1080, 1964.
- REILEY, M. & GOCHMAN, N.: A fully automated method for the determination of the serum protein-bound iodine. Technical Symposium 1964, New York.
- RERUP, C. & MELANDER, A.: *Acta Endocr.* 50:177, 1965.
- ROITT, I.M. & DONIACH, D.: *Lancet* II:1027, 1958.
- ROSE, N.R., KITE, J.H. & DOEBLER, T.K. In: Mechanisms of cell and tissue damage produced by immune reactions. (eds. Grabor, P. & Miescher, P.) p. 161. Schwabe Basel 1962.
- SANDRITTER, W. & KLEIN, K.H.: *Frankfurt. Z. Path.* 65:204, 1954.
- SCHADE, R.O.K., OWEN, S.G., SMART, G.A. & HALL, R.: *J. Clin. Path.* 13:499, 1960.
- SIVULA, A.: *Ann. Chir. et Gyn. Fenn.*: 58:130, 1969.
- SMITH, R.N. & LALJEE, H.C.K.: *Br. M. J.* 4:589, 1967.
- SOKAL, E.: *JAMA* 154:1321, 1954.
- STERLING, K., REFETOFF, S. & SELENKOW, H.A.: *JAMA* 213:571, 1970.
- SUGIYAMA, S.: *Erg. Anat. Entw. Gesch. Bd* 39, Heft 3, 1967.
- SÖDERSTRÖM, N.: *Acta med. Scand.* 144:237, 1952.
- SÖDERSTRÖM, N.: Fine-Needle Aspiration Biopsy Used as a Direct Adjunct in Clinical Diagnostic Work. Almkvist & Wiksell, Stockholm 1966.
- TASHJIAN, A.H. & VOELKEL, E.F.: *J. Clin. Endocr.* 27:1353, 1967.
- TASHJIAN, A.H. & MELVIN, K.E.W.: *New Engl. J. Med.* 279:279, 1968.
- TASHJIAN, A.H., HOWLAND, B.G., MELVIN, K.E.W. & HILL, C.S.: *New Engl. J. Med.* 283:890, 1970.
- VENDRELY, R. & VENDRELY, C.: *Int. Rev. Cyt.* 5:171, 1955.
- WERNER, S.C. & PLATMAN, S.R.: *Lancet* 2:751, 1965.
- WARTHIN, A.S.: *Ann. Int. Med.* 2:553, 1928.
- WHITESSELL, F.B. & BLACK, B.M.: *J. Clin. Endocr.* 9:1202, 1949.
- WILLIAMS, E.D.: Medullary Carcinoma of the Thyroid. In: Calcitonin 1969. Proc. 2nd Internat. Symp. (ed. S. Taylor) p. 483. Heinemann, London 1970.

WOLF, W.: Endocrinology in modern practice p. 258. Saunders Comp.
London 1937.

The work was supported by grants from Axel Linder's foundation.

